Conclusion. The sodium channel $Na_V1.8$ is expressed in murine heart, and is functionally present in intracardiac neurons, but absent in cardiomyocytes. Thus, $Na_V1.8$ may influence myocardial electrophysiological properties through its contribution to cardiac neuronal activity.

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Turret Histidines in pH Modulation of the Cardiac Voltage-Gated Sodium Channel
David K. Jones, Thomas W. Claydon, Peter C. Ruben.
Low pH reduces single channel conductance and destabilizes the inactivated states of the cardiac voltage gated sodium channel (NaV1.5) by increasing window current and inhibiting use dependent inactivation. Outer ring carboxylates are believed to underlie proton block. In contrast, the molecular underpinnings by which protons modulate NaV1.5 channel kinetics (Jones et al. 2010) are unknown. We hypothesize that turret histidines found in domain II, and conserved in all NaV channel isoforms, play a pivotal role in pH modulation and channel function, as has been demonstrated in potassium channels (Claydon et al. 2002). We expressed wild type and mutant NaV1.5 channels in Xenopus oocytes and recorded currents using a cut-open voltage clamp with extracellular solution titrated to either pH 7.4 or pH 6.0. Replacement of a histidine with a glutamine at position 880 abolished the effect of pH on window current and use dependent inactivation. Additionally, the H880Q mutant depolarized the voltage dependence of activation and fast inactivation. We therefore propose H880 plays an important role in pH modulation and function of NaV1.5.

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Palmitoylation Affects the Interaction of Animal Toxins with Sodium Channels
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Animal toxins can interact with voltage sensor paddle motifs within voltage-activated sodium (Nav) channel voltage sensors to alter channel function. These structural motifs were originally identified in voltage-activated potassium (Kv) channels, where they were shown to move at the protein-lipid interface to drive activation of the voltage sensors and opening of the pore. Serendipitously, we identified a variant of Nav1.2 with functional properties similar to wild-type, but that exhibits a 20-fold higher apparent affinity for PaurTx3, a voltage sensor toxin isolated from tarantula venom. The amino acid difference that underlies this large divergence in toxin sensitivity was identified as G1079C and is located in the intracellular loop between domain II and domain III of the channel. Since this region is unlikely to be directly accessible to peptide toxins applied to the external solution, we explored the possibility that cysteine palmitoylation underlies this observation. When we inhibit palmitoylation of Nav1.2 and the G1079C mutant by using 2-Br-palmitate, the apparent affinities of the toxin for both channels now coincide. While surveying other molecules, we found a second tarantula toxin, ProTx-II, which is similarly influenced by Nav1.2 palmitoylation. Interestingly, both these toxins interact with the paddle motif in domain II of the channel whereas a scorpion toxin that interacts with the domain IV voltage sensor, AaHII, is not affected. These results suggest an important role for palmitoylation in shaping the pharmacological properties of Nav1.2 and have implications for changes in drug susceptibility of Nav channels caused by intracellular mutations.

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Molecular Determinants for Alpha-Scorpion Toxin Binding to the Resting State of a Voltage Sensor of Brain Sodium Channels
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Voltage-gated sodium channels are responsible for initiation and propagation of action potentials in nerve and muscle. α-Scorpion toxins, including LqhII (*Leiurus quinquestriatus hebraeus, type II*), bind to the extracellular face of the sodium channel and inhibit fast inactivation. Previous work has identified E1613 (rat $Na_V1.2$) in the S3-S4 loop of domain IV as a key site of toxin binding, but antibody-mapping studies suggested additional components of the toxin receptor site in domains I and IV. To identify other determinants of toxin action, extracellular amino acid residues in domains I and IV of the α subunit were converted to alanine or other neutral amino acids, and mutant channels were transiently expressed in tsA-201 cells and tested for channel function and toxin action by whole-cell voltage clamp. Toxin affinity for most mutant channels was unchanged from wild-type. However, electrophysiological analysis showed that T393A, T1560A, F1610A, and E1613A, had 3.4-, 5.9-, 10.7-, and 3.9-fold lower affinities for LqhII, respectively. Most of these mutations increased the rate of toxin dissociation, but had little effect on the rate of toxin association. These results indicate that T1560 in the S1-S2 loop, F1610 in the S3 segment, and E1613 in the S3-S4 loop in domain IV form one aspect of the toxin-binding site; T393 in the domain I SS2-S6 loop may contribute to a second part of the site. Modeling the toxin receptor site and the toxin-receptor interaction with the Rosetta-Membrane structural-modeling and ligand-docking algorithms resulted in a three-dimensional model of LqhII binding to the voltage sensor of the channel. This model refines the structure of the resting state of the voltage sensor and suggests its mode of interaction with a gating modifier toxin. Supported by U01 NS058039.

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High-Resolution Structural Modeling of Voltage-Dependent Conformational Changes in the Voltage Sensor of NaChBac
Vladimir M. Yarov-Yarovoy, Paul DeCaen, Todd Scheuer, David Baker, William A. Catterall.
Progress has been made in determining high-resolution structures of voltage sensors of voltage-gated ion channels in activated and/or inactivated states. However, high-resolution structures of resting and intermediate states of voltage sensors remain unknown. We constructed high-resolution structural models of resting, intermediate, and activated states of the voltage-sensing domain (VSD) of the bacterial sodium channel NaChBac using the Rosetta-Membrane computational method, the Rosetta method-based FoldIt program, the x-ray structure of the Kv1.2-Kv2.1 chimeric channel, and experimental data demonstrating sequential interactions between gating-charge-carrying arginines in S4 segment and negatively charged residues in S1, S2, and S3 segments during activation. The resulting sliding helix model suggests that the S4 is a 3-10 helix from the first or second gating-charge-carrying arginine to the fourth gating-charge-carrying arginine or S125 near its C-terminus during the conformational change between the resting and activated states. The S4 segment slides ~10 Å through a narrow groove formed by rigid S1, S2 and S3 segments, rotates ~30-60 degrees with respect to its own axis, and tilts sideways at a pivot point formed by a highly conserved hydrophobic region in the middle of the VSD. During S4 movement, gating-charge-carrying arginines sequentially form ion pairs and hydrogen bonds with highly conserved negatively charged and polar residues in the narrow gating pore and in the intracellular and extracellular water-accessible cavities of the VSD. Conformational changes of the intracellular half of S4 are coupled to lateral movement of the S4-S5 linker that leads to movement of the intracellular half of S5 and S6 segments and either opens or closes the intracellular gate of the ion-conducting pore. Supported by NIH R01 NS015751 to W.A.C. and P20GM076222 to D.B.

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Disulfide Locking Reveals Interaction of the Gating Charges with a Negative Charge in the S1 Segment During Activation of the NaChBac Voltage Sensor
Paul G. DeCaen, Vladimir Yarov-Yarovoy, Todd Scheuer, William A. Catterall.
Opening and closing the pore of voltage-gated ion channels are mechanically linked to conformational movement of the positively charged fourth transmembrane segment (S4) in the voltage sensor. Disulfide locking of cysteines substituted for gating charges and for E43 at the extracellular end of the S1 segment of the bacterial sodium channel NaChBac demonstrated that a threonine at gating charge position 0 (T0) and arginine at the first gating charge position (R1) located at the extracellular end of S4 interact with E43 in the closed state, whereas the second and the third gating charges (R2) and (R3) interact with E43 in activated states. The kinetics and voltage dependence of disulfide locking of R2C and R3C demonstrate sequential interactions of the gating charges with E43C during voltage sensor activation. We did not observe disulfide locking with the fourth or last gating charge (R4) and E43, suggesting that we have described the upper limit to the outward movement made by the S4 during channel activation. These results indicate that S4 moves 8-10Å outward with respect to E43 in the transition from resting to activate states, which supports a sliding helix model of voltage sensor movement in voltage-gated ion channels.

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An Alternative Tetramerisation Domain Restores Expression of the NAChBac Voltage-Gated Sodium Channel
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The NaChBac voltage-gated sodium channel from Bacillus halodurans is a homo-tetramer, which matches the quaternary structure of potassium channels but contrasts with the single-chain mammalian sodium channels. We have previously demonstrated that the cytoplasmic C-terminus of NaChBac functions as a channel assembly domain [Powl et al (2010) PNAS 107:14064-14069]. Sequential deletion mutations reduce the amount of NaChBac in the membrane fraction, with removal of the entire C-terminus eliminating the channel assembly. A similar effect was also found upon removal of potassium channel assembly domains. However, potassium channel expression can be rescued by using an alternative tetramerisation domain replacement.
In this study we generated a chimeric form of NaChBac in which the C-terminus was removed and the short cytoplasmic N-terminus was replaced with the